Introduction

Post-traumatic stress disorder (PTSD) and substance use disorder (SUD) are highly comorbid, with estimates as high as 50-60% comorbidity in veterans. Our laboratory has shown that comorbidity can be modeled in rats utilizing an acute stressor, repeated unsignaled footshock, with shocked rats showing an increased sensitivity to drug-related cues, higher baseline anxiety, and increased fear expression following mild stress in a new context.

Numerous experiments have shown that Pavlovian conditioning plays a significant role in mediating drug relapse. To test for the ability of an acute stress to influence drug-seeking, we used a well-characterized model of substance use, drug self-administration. Rats infused methamphetamine intravenously for 2 weeks following acute stressor, repeated unsignaled footshock, with shocked rats showing an increase in lever pressing with a drug-related context and the unconditioned stimulus of saline.

Mice were used in a conditioned place preference (CPP) study concurrent with illumination of a cue light above the active lever. A second intraperitoneal administration of methamphetamine 60 days later did not lead to CPP to a drug-paired cue light to initiate drug seeking when then assessed.

Mice were used in a conditioned place preference (CPP) study. CPP induces a Pavlovian association between a context and the conditioned stimulus of a drug. Preference for a drug-paired context versus a neutral context is used to assess how reinforcing that drug is for the animal, with pleasurable drugs leading to CPP and unpleasant drugs leading to the avoidance of the paired floor.

Materials and Methods

Subjects

16 male Long-Evans rats were utilized for our experiments, with those self-administering meth undergoing surgery to place a catheter in their right jugular veins for IV infusions. 24 male C57Bl/6 mice were used for CPP experiments. Rats and mice were approximately 15-12 weeks old at the start of each session.

Apparatus & Procedure

Fear conditioning occurred in a novel context, in a separate room from any appetitive conditioning. Rats were given 1 shock for 1 second in duration. CPP procedure was 15 unsignaled footshocks over 60 minutes, pseudorandom variable inter-stimulus interval with an average of 6 minutes between shocks.

Opener Self-Administration

At the start of each session, two levers would extend. One would be randomly designated the active lever prior to the start of the experiment, and would remain the active lever for all sessions.

Pressing of the active lever would lead to intravenous methamphetamine infusion, as well as the lighting of a cue light directly above the active lever. Infusion and cue-light would continue for 3 seconds.

Conditioned Place Preference

Over 8 days, mice received 4 pairings of cocaine and 4 pairings of saline with a floor type (restricted to that floor for the conditioning trial). After 4 pairings of cocaine and saline on their respective floors, mice were given either 15 or 15 footshocks in Context A, but those that received 4 or 15 footshocks in Context B were footshocked more frequently. Q) Shock and avoidance of shock were long-lasting, remaining at least 60 days since original shock in Context A.*** p<0.01 compared to 1 shocks in Context A, *** p<0.01 compared to 1 shocks.

Conclusions/Future Directions

Acute stress in the form of unsignaled footshock leads to long-term changes in behavior in animals, including an increased sensitivity to future fearful events, increases in anxiety, and enhancements in the ability of drug-paired cues to initiate drug-seeking. While there is an increase in stress response (as assayed by corticosterone levels), this increase is only in response to the acute stress and not due to increased HPA axis activity or dysfunction. It remains unclear what drives these long-term changes after a traumatic event, whether these differing behavioral outcomes have a similar neurological locus, and what predisposes some to PTSD while others show resilience.

Future work will look at the acquisition of these symptoms (preoccupation with drug abuse, anxiety, and hyper-responsiveness to perceived threats) and how trauma induces such behavior. A strong candidate region is the basolateral amygdala (BLA), which is required for the acquisition and expression of all of these behaviors. Future work will involve selective manipulation of this region during acute stress to attempt to disassociate what role the BLA plays in each allostressed behavior. Additionally, future work will look at alcohol in particular (the most widely abused drug for PTSD patients) as an abused substance, and whether acute stress shows similar effects with alcohol self-administration.

Preliminary data suggests that shock instead affects the amount of alcohol preferred and the willingness to work for it (below), rather than a response to alcohol-related cues, but more research is required.

Effect of Shock on Operant Behavior for Fear

Acute stress induces corticosterone release without noticeable HPA axis dysregulation. Rats were given either 15 or 0 footshocks in Context A and then had blood drawn for CORT analysis. CORT was only significantly different on the day of shock. A decrease in corticosterone (a synthetic glucocorticoid) suppression test revealed normal negative feedback of the HPA axis, indicating to HPA axis dysregulation.

Acute stress enhances cues associated with drug-seeking

Shock induces long-term enhancement of fear

Shock causes an anxious phenotype

Acute stress enhances response to drug-paired cues

Figure 1. Schematic of the SEFL procedure. Rats received either 10 or 0 footshocks in Context A, and were tested for freezing behavior 24 hours later. One day after that, all groups receive a single shock in a new context, Context B. Rats in Context A receive fear conditioning in a novel context, in a separate room from any appetitive conditioning.

Figure 2. Acute stress induces anxiety as measured by elevated plus maze. Two of elevated plus maze made over 5 minutes, one week after shock in Context A. Rats that had received 16 footshocks showed significantly more anxiety than those rats that had not been shocked.

Figure 3. Effect of shock on EPM performance

Figure 4. Acute stress enhances response to drug-paired cues. A) Schematic of timeline for methamphetamine self-administration experiment. B) Effect of footshocks on a different context prior to acquisition of meth self-administration doesn’t increase acquisition or impact extinction of drug-seeking. C) but does enhance responding to a cue that was paired with methamphetamine infusions, p<0.05.

Figure 5. Mice show an increased preference for a drug-paired floor after shock. Mice were given interperitoneal injections of either cocaine (CS+) or saline (CS-) paired with either a grid or a hole in type floor (restricted to that floor for the conditioning trial). After 4 pairings of cocaine and saline on their respective floors, mice were given either 15 or 0 footshocks in a different context. Immediately after fear conditioning, mice were returned back in the CPP apparatus and allowed to spend time on either the CS- or CS+ floor. Shock enhanced preference for the cocaine paired floor on both the day of shock and one day later (p<0.05).

References/Founding

Achievement Rewards for College Scientists (ARCS) Foundation—Harold Snitzberger Scholar

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